

## **METHODS AND COMPOSITIONS FOR TREATING NEPHROTIC SYNDROME**

### **FIELD OF THE INVENTION**

The invention relates to yeast compositions that can ameliorate or  
5 prevent nephrotic syndrome and are useful as a dietary supplement (e.g., health  
drink) or medication. These compositions contain yeast cells obtainable by growth  
in electromagnetic fields with specific frequencies and field strengths.

### **BACKGROUND OF THE INVENTION**

Nephrotic syndrome is a condition caused by a group of diseases  
10 that damage the kidney's filtering system, the glomeruli. The two main features of  
nephrotic syndrome are excess excretion of proteins in the urine (proteinuria) and  
lower level of protein in the blood (hypoalbuminemia). Other major symptoms  
include swelling (edema) and high level of cholesterol in the blood  
(hypercholesterolemia).

15 Nephrotic syndrome may be caused by both kidney diseases and  
non-kidney diseases, such as diabetes, lupus and hypertension. Primary causes  
include minimal change disease, focal segmental glomerulosclerosis, membranous  
glomerulonephritis, membranoproliferative glomerulonephritis and mesangial  
proliferative glomerulonephritis.

20 Nephrotic syndrome is usually diagnosed by clinical testing and  
confirmed by renal biopsy. An initial urinalysis is done to measure the amount of  
protein in the urine by collecting urine for 24 hours. A blood test is commonly

done to detect the protein, cholesterol and triglyceride levels in the blood. It is common to have abnormal blood overclots (coagulopathies) due to the urinary loss of certain protein in patients with nephrotic syndrome. A blood test may also be used to detect serum levels of factor VIII, fibrinogen and platelets.

5                    Treatment of nephrotic syndrome is directed at the underlying disease. Some of the diseases that cause nephrotic syndrome can be treated with medication. Some do not require treatment and will get better on their own. However, many of the underlying diseases causing nephrotic syndrome have no treatment. There remains a need for an effective treatment for nephrotic syndrome.

## 10                    **SUMMARY OF THE INVENTION**

                    This invention is based on the discovery that certain yeast cells can be activated by electromagnetic fields having specific frequencies and field strengths to produce substances useful in treating nephrotic syndrome. Compositions comprising these activated yeast cells can therefore be used as a  
15   medication or dietary supplement, in the form of health drinks or dietary pills (tablets or powder). For instance, these compositions can be used to alleviate nephrotic syndrome (e.g., lower urinary protein and increase serum protein) in animals (including humans), or to prevent or postpone the onset of nephrotic syndrome in a high risk individual (e.g., someone predisposed to nephrotic  
20   syndrome because of his health or life style).

                    This invention embraces a composition comprising a plurality of yeast cells that have been cultured in an alternating electric field having a frequency in the range of about 9500 to 13500 MHz (e.g., 9700-10700 and 11800-12800 MHz) and a field strength in the range of about 250 to 600 mV/cm (e.g., 285-305,  
25   285-315, 320-350, 325-355, 340-370, 360-390, 400-440, 410-450, 430-470, 440-480, 460-500 and 480-520 mV/cm). The yeast cells are cultured for a period of time sufficient to activate said plurality of yeast cells to produce substances useful in treating nephrotic syndrome in a subject. In one embodiment, the frequency and/or the field strength of the alternating electric field can be altered within the  
30   aforementioned ranges during said period of time. In other words, the yeast cells

are exposed to a series of electromagnetic fields. An exemplary period of time is about 20-150 hours (e.g., 40-130 hours).

Also included in this invention is a composition comprising a plurality of yeast cells that have been cultured under acidic conditions in an alternating electric field having a frequency in the range of about 12000 to 13000 MHz (e.g., 12500-12700 MHz) and a field strength in the range of about 250 to 450 mV/cm (e.g., 360-390 or 285-315 mV/cm). In one embodiment, the yeast cells are exposed to a series of electromagnetic fields. An exemplary period of time is about 20-80 hours (e.g., 30-70 hours).

10 Included in this invention are also methods of making the above compositions.

Yeast cells that can be included in this composition can be derived from parent strains publically available from the China General Microbiological Culture Collection Center ("CGMCC"), China Committee for Culture Collection of Microorganisms, Institute of Microbiology, Chinese Academy of Sciences, Haidian, P.O. Box 2714, Beijing, 100080, China. Useful yeast species include, but are not limited to, those commonly used in food and pharmaceutical industries, such as *Saccharomyces cerevisiae*, *Saccharomyces carlsbergensis*, *Saccharomyces rouxii*, *Saccharomyces sake*, *Saccharomyces uvarum*, *Saccharomyces sp.*, *Schizosaccharomyces pombe*, or *Rhodotorula aurantiaca*. For instance, the yeast cells can be of the strain *Saccharomyces cerevisiae* Hansen AS2.502, IFFI1010 or AS2.53, *Saccharomyces sake* Yabe ACCC2045, *Saccharomyces uvarum* Beijer IFFI1072, or *Schizosaccharomyces pombe* Lindner AS2.248. Other useful yeast strains are illustrated in Table 1.

25 This invention further embraces a composition comprising a plurality of yeast cells, wherein said plurality of yeast cells have been activated to treat nephrotic syndrome in a subject. Included in this invention are also methods of making these compositions.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those

described herein can also be used in the practice or testing of the present invention. All publications and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. The materials, methods, and examples are illustrative  
5 only and not intended to be limiting. Throughout this specification and claims, the word “comprise,” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. A subject includes a human and veterinary subject.

10 Other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 is a schematic diagram showing an exemplary apparatus for activating yeast cells using electromagnetic fields. 1: yeast culture; 2: container;  
15 3: power supply.

Fig. 2 is a schematic diagram showing an exemplary apparatus for making yeast compositions of the invention. The apparatus comprises a signal generator and interconnected containers A, B and C.

### **DETAILED DESCRIPTION OF THE INVENTION**

20 This invention is based on the discovery that certain yeast strains can be activated by electromagnetic fields (“EMF”) having specific frequencies and field strengths to become highly efficient in producing substances that alleviate nephrotic syndrome in a subject. Compositions containing these activated yeast cells are therefore useful in the treatment of nephrotic syndrome, e.g., in decreasing  
25 urinary protein and/or increasing serum protein levels. Yeast compositions containing activated yeast cells can be used as medication, or as a dietary supplements, in the form of health drinks or dietary pills (tablets or powder).

Since the activated yeast cells contained in the yeast compositions have been cultured to endure acidic conditions (pH 2.5-4.2), these cells can survive  
30 the gastric environment and pass on to the intestines. Once in the intestines, the

yeast cells are ruptured by various digestive enzymes, and the useful substances for treatment of nephrotic syndrome are released and readily absorbed.

**I. Yeast Strains Useful in the Invention**

The types of yeasts useful in this invention include, but are not  
5 limited to, yeasts of the genera *Saccharomyces*, *Schizosaccharomyces pombe* and *Rhodotorula*.

Exemplary species within the above-listed genera include, but are not limited to, those illustrated in Table 1. Yeast strains useful for this invention can be obtained from laboratory cultures, or from publically accessible culture  
10 depositories, such as CGMCC and the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209. Non-limiting examples of useful strains (with accession numbers of CGMCC) are *Saccharomyces cerevisiae* Hansen AS2.502, IFFI1010 and AS2.53, *Saccharomyces sake* Yabe ACCC2045, *Saccharomyces uvarum* Beijer IFFI1072 and *Schizosaccharomyces pombe* Lindner  
15 AS2.248. Other useful yeast strains are illustrated in Table 1.

The preparation of the yeast compositions of this invention is not limited to starting with a pure strain of yeast. A yeast composition of the invention may be produced by culturing a mixture of yeast cells of different species or strains. The ability of any activated species or strain of yeasts to treat nephrotic  
20 syndrome can be readily tested by methods known in the art. See, for instance, Examples 1 and 2.

Table 1 Exemplary Yeast Strains

<i>Saccharomyces cerevisiae</i> Hansen					
	ACCC2034	ACCC2035	ACCC2036	ACCC2037	ACCC2038
	ACCC2039	ACCC2040	ACCC2041	ACCC2042	AS2. 1
5	AS2. 4	AS2. 11	AS2. 14	AS2. 16	AS2. 56
	AS2. 69	AS2. 70	AS2. 93	AS2. 98	AS2. 101
	AS2. 109	AS2. 110	AS2. 112	AS2. 139	AS2. 173
	AS2. 174	AS2. 182	AS2. 196	AS2. 242	AS2. 336
	AS2. 346	AS2. 369	AS2. 374	AS2. 375	AS2. 379
10	AS2. 380	AS2. 382	AS2. 390	AS2. 393	AS2. 395
	AS2. 396	AS2. 397	AS2. 398	AS2. 399	AS2. 400
	AS2. 406	AS2. 408	AS2. 409	AS2. 413	AS2. 414
	AS2. 415	AS2. 416	AS2. 422	AS2. 423	AS2. 430
	AS2. 431	AS2. 432	AS2. 451	AS2. 452	AS2. 453
15	AS2. 458	AS2. 460	AS2. 463	AS2. 467	AS2. 486
	AS2. 501	AS2. 502	AS2. 503	AS2. 504	AS2. 516
	AS2. 535	AS2. 536	AS2. 558	AS2. 560	AS2. 561
	AS2. 562	AS2. 576	AS2. 593	AS2. 594	AS2. 614
	AS2. 620	AS2. 628	AS2. 631	AS2. 666	AS2. 982
20	AS2. 1190	AS2. 1364	AS2. 1396	IFFI1001	IFFI1002
	IFFI1005	IFFI1006	IFFI1008	IFFI1009	IFFI1010
	IFFI1012	IFFI1021	IFFI1027	IFFI1037	IFFI1042
	IFFI1043	IFFI1045	IFFI1048	IFFI1049	IFFI1050
	IFFI1052	IFFI1059	IFFI1060	IFFI1062	IFFI1063
25	IFFI1202	IFFI1203	IFFI1206	IFFI1209	IFFI1210
	IFFI1211	IFFI1212	IFFI1213	IFFI1214	IFFI1215
	IFFI1220	IFFI1221	IFFI1224	IFFI1247	IFFI1248
	IFFI1251	IFFI1270	IFFI1277	IFFI1287	IFFI1289
	IFFI1290	IFFI1291	IFFI1292	IFFI1293	IFFI1297
30	IFFI1300	IFFI1301	IFFI1302	IFFI1307	IFFI1308
	IFFI1309	IFFI1310	IFFI1311	IFFI1331	IFFI1335

	IFFI1336	IFFI1337	IFFI1338	IFFI1339	IFFI1340
	IFFI1345	IFFI1348	IFFI1396	IFFI1397	IFFI1399
	IFFI1411	IFFI1413	IFFI1441	IFFI1443	
	<i>Saccharomyces cerevisiae</i> Hansen Var. ellipsoideus (Hansen) Dekker				
5	ACCC2043	AS2.2	AS2.3	AS2.8	AS2.53
	AS2.163	AS2.168	AS2.483	AS2.541	AS2.559
	AS2.606	AS2.607	AS2.611	AS2.612	
	<i>Saccharomyces chevalieri</i> Guilliermond				
	AS2.131	AS2.213			
10	<i>Saccharomyces delbrueckii</i>				
	AS2.285				
	<i>Saccharomyces delbrueckii</i> Lindner ver. mongolicus (Saito) Lodder et van Rij				
	AS2.209	AS2.1157			
	<i>Saccharomyces exiguus</i> Hansen				
15	AS2.349	AS2.1158			
	<i>Saccharomyces fermentati</i> (Saito) Lodder et van Rij				
	AS2.286	AS2.343			
	<i>Saccharomyces logos</i> van laer et Denamur ex Jorgensen				
	AS2.156	AS2.327	AS2.335		
20	<i>Saccharomyces mellis</i> (Fabian et Quinet) Lodder et kreger van Rij				
	AS2.195				
	<i>Saccharomyces mellis</i> Microellipsoides Osterwalder				
	AS2.699				
	<i>Saccharomyces oviformis</i> Osteralder				
25	AS2.100				
	<i>Saccharomyces rosei</i> (Guilliermond) Lodder et Kreger van Rij				
	AS2.287				
	<i>Saccharomyces rouxii</i> Boutroux				
	AS2.178	AS2.180	AS2.370	AS2.371	

	<i>Saccharomyces sake</i> Yabe				
	ACCC2045				
	<i>Candida arborea</i>				
	AS2.566				
5	<i>Candida lambica</i> (Lindner et Genoud) van. Uden et Buckley				
	AS2.1182				
	<i>Candida krusei</i> (Castellani) Berkhout				
	AS2.1045				
	<i>Candida lipolytica</i> (Harrison) Diddens et Lodder				
10	AS2.1207	AS2.1216	AS2.1220	AS2.1379	AS2.1398
	AS2.1399	AS2.1400			
	<i>Candida parapsilosis</i> (Ashford) Langeron et Talice Var. intermedia Van Rij et Verona				
	AS2.491				
15	<i>Candida parapsilosis</i> (Ashford) Langeron et Talice				
	AS2.590				
	<i>Candida pulcherrima</i> (Lindner) Windisch				
	AS2.492				
	<i>Candida rugosa</i> (Anderson) Diddens et Lodder				
20	AS2.511	AS2.1367	AS2.1369	AS2.1372	AS2.1373
	AS2.1377	AS2.1378	AS2.1384		
	<i>Candida tropicalis</i> (Castellani) Berkhout				
	ACCC2004	ACCC2005	ACCC2006	AS2.164	AS2.402
	AS2.564	AS2.565	AS2.567	AS2.568	AS2.617
25	AS2.637	AS2.1387	AS2.1397		
	<i>Candida utilis</i> Henneberg Lodder et Kreger Van Rij				
	AS2.120	AS2.281	AS2.1180		
	<i>Crebrothecium ashbyii</i> (Guilliermond) Routein ( <i>Eremothecium ashbyii</i> Guilliermond)				
30	AS2.481	AS2.482	AS2.1197		



	<i>Geotrichum candidum</i> Link				
	ACCC2016	AS2.361	AS2.498	AS2.616	AS2.1035
	AS2.1062	AS2.1080	AS2.1132	AS2.1175	AS2.1183
	<i>Hansenula anomala</i> (Hansen)H et P sydow				
5	ACCC2018	AS2.294	AS2.295	AS2.296	AS2.297
	AS2.298	AS2.299	AS2.300	AS2.302	AS2.338
	AS2.339	AS2.340	AS2.341	AS2.470	AS2.592
	AS2.641	AS2.642	AS2.782	AS2.635	AS2.794
	<i>Hansenula arabitolgens</i> Fang				
10	AS2.887				
	<i>Hansenula jadinii</i> (A. et R Sartory Weill et Meyer) Wickerham				
	ACCC2019				
	<i>Hansenula saturnus</i> (Klocker) H et P sydow				
	ACCC2020				
15	<i>Hansenula schneeggii</i> (Weber ) Dekker				
	AS2.304				
	<i>Hansenula subpelliculosa</i> Bedford				
	AS2.740	AS2.760	AS2.761	AS2.770	AS2.783
	AS2.790	AS2.798	AS2.866		
20	<i>Kloeckera apiculata</i> (Reess emend. Klocker) Janke				
	ACCC2022	ACCC2023	AS2.197	AS2.496	AS2.714
	ACCC2021	AS2.711			
	<i>Lipomycess starkeyi</i> Lodder et van Rij				
	AS2.1390	ACCC2024			
25	<i>Pichia farinosa</i> (Lindner) Hansen				
	ACCC2025	ACCC2026	AS2.86	AS2.87	AS2.705
	AS2.803				
	<i>Pichia membranaefaciens</i> Hansen				
	ACCC2027	AS2.89	AS2.661	AS2.1039	
30	<i>Rhodospiridium toruloides</i> Banno				

	ACCC2028				
	<i>Rhodotorula glutinis</i> (Fresenius) Harrison				
	AS2.2029	AS2.280	ACCC2030	AS2.102	AS2.107
	AS2.278	AS2.499	AS2.694	AS2.703	AS2.704
5	AS2.1146				
	<i>Rhodotorula minuta</i> (Saito) Harrison				
	AS2.277				
	<i>Rhodotorula rubra</i> (Demme) Lodder				
	AS2.21	AS2.22	AS2.103	AS2.105	AS2.108
10	AS2.140	AS2.166	AS2.167	AS2.272	AS2.279
	AS2.282	ACCC2031			
	<i>Rhodotorula aurantiaca</i> (Saito) Lodder				
	AS2.102	AS2.107	AS2.278	AS2.499	AS2.694
	AS2.703	AS2.1146			
15	<i>Saccharomyces carlsbergensis</i> Hansen				
	AS2.113	ACCC2032	ACCC2033	AS2.312	AS2.116
	AS2.118	AS2.121	AS2.132	AS2.162	AS2.189
	AS2.200	AS2.216	AS2.265	AS2.377	AS2.417
	AS2.420	AS2.440	AS2.441	AS2.443	AS2.444
20	AS2.459	AS2.595	AS2.605	AS2.638	AS2.742
	AS2.745	AS2.748	AS2.1042		
	<i>Saccharomyces uvarum</i> Beijer				
	IFFI1023	IFFI1032	IFFI1036	IFFI1044	IFFI1072
	IFFI1205	IFFI1207			
25	<i>Saccharomyces willianus</i> Saccardo				
	AS2.5	AS2.7	AS2.119	AS2.152	AS2.293
	AS2.381	AS2.392	AS2.434	AS2.614	AS2.1189
	<i>Saccharomyces</i> sp.				
	AS2.311				
30	<i>Saccharomycodes ludwigii</i> Hansen				

	ACCC2044	AS2.243	AS2.508	
	<i>Saccharomyces sinenses</i> Yue			
	AS2.1395			
	<i>Schizosaccharomyces octosporus</i> Beijerinck			
5	ACCC2046	AS2.1148		
	<i>Schizosaccharomyces pombe</i> Lindner			
	ACCC2047	ACCC2048	AS2.214	AS2.248 AS2.249
	AS2.255	AS2.257	AS2.259	AS2.260 AS2.274
	AS2.994	AS2.1043	AS2.1149	AS2.1178 IFFI1056
10	<i>Sporobolomyces roseus</i> Kluyver et van Niel			
	ACCC2049	ACCC2050	AS2.19	AS2.962 AS2.1036
	ACCC2051	AS2.261	AS2.262	
	<i>Torulopsis candida</i> (Saito) Lodder			
	AS2.270	ACCC2052		
15	<i>Torulopsis famta</i> (Harrison) Lodder et van Rij			
	ACCC2053	AS2.685		
	<i>Torulopsis globosa</i> (Olson et Hammer) Lodder et van Rij			
	ACCC2054	AS2.202		
	<i>Torulopsis inconspicua</i> Lodder et Kreger van Rij			
20	AS2.75			
	<i>Trichosporon behrendii</i> Lodder et Kreger van Rij			
	ACCC2056	AS2.1193		
	<i>Trichosporon capitatum</i> Diddens et Lodder			
	ACCC2056	AS2.1385		
25	<i>Trichosporon cutaneum</i> (de Beurm et al.) Ota			
	ACCC2057	AS2.25	AS2.570	AS2.571 AS2.1374
	<i>Wickerhamia fluorescens</i> (Soneda) Soneda			
	ACCC2058	AS2.1388		

## II. Application of Electromagnetic Fields

An electromagnetic field useful in this invention can be generated and applied by various means well known in the art. For instance, the EMF can be generated by applying an alternating electric field or an oscillating magnetic field.

5 Alternating electric fields can be applied to cell cultures through electrodes in direct contact with the culture medium, or through electromagnetic induction. See, e.g., Fig. 1. Relatively high electric fields in the medium can be generated using a method in which the electrodes are in contact with the medium. Care must be taken to prevent electrolysis at the electrodes from introducing  
10 undesired ions into the culture and to prevent contact resistance, bubbles, or other features of electrolysis from dropping the field level below that intended. Electrodes should be matched to their environment, for example, using Ag-AgCl electrodes in solutions rich in chloride ions, and run at as low a voltage as possible. For general review, see Goodman et al., *Effects of EMF on Molecules and Cells*,  
15 International Review of Cytology, A Survey of Cell Biology, Vol. 158, Academic Press, 1995.

The EMFs useful in this invention can also be generated by applying an oscillating magnetic field. An oscillating magnetic field can be generated by oscillating electric currents going through Helmholtz coils. Such a magnetic field  
20 in turn induces an electric field.

The frequencies of EMFs useful in this invention range from about 9500 to 13500 MHz (e.g., 9700-10700 and 11800-12800 MHz). Exemplary frequencies are 10156, 10185, 12107, 12687 and 12698 MHz. The field strength of the electric field useful in this invention ranges from about 250 to 600 mV/cm (e.g.,  
25 285-305, 285-315, 320-350, 325-355, 340-370, 360-390, 400-440, 410-450, 430-470, 440-480, 460-500 and 480-520 mV/cm). Exemplary field strengths are 296, 332, 353, 364, 373, 416, 435, 443, 456, 487 and 507 mV/cm.

When a series of EMFs are applied to a yeast culture, the yeast culture can remain in the same container while the same set of EMF generator and  
30 emitters is used to change the frequency and/or field strength. The EMFs in the series can each have a different frequency or a different field strength; or a different frequency and a different field strength. Such frequencies and field strengths are

preferably within the above-described ranges. Although any practical number of EMFs can be used in a series, it may be preferred that the yeast culture be exposed to, for example, a total of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or more EMFs in a series. In one embodiment, the yeast culture is exposed to a series of EMFs,  
5 wherein the frequency of the electric field is alternated in the range of 9700-10700 and 11800-12800 MHz.

Although the yeast cells can be activated after even a few hours of culturing in the presence of an EMF, it may be preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EMF(s) for a total of  
10 20-150 hours (e.g., 40-120 hours).

Fig. 1 illustrates an exemplary apparatus for generating alternating electric fields. An electric field of a desired frequency and intensity is generated by an AC source (3) capable of generating an alternating electric field, preferably in a sinusoidal wave form, in the frequency range of 10 to 20,000 MHz. Signal  
15 generators capable of generating signals with a narrower frequency range can also be used. If desirable, a signal amplifier can also be used to increase the output. The activation container (2) can be made from non-conductive material, e.g., plastics, glass or ceramic. The wire connecting the activation container (2) and the signal generator (3) is preferably a high frequency coaxial cable with a transmission  
20 frequency of at least 30 GHz.

The alternating electric field can be applied to the culture by a variety of means, including placing the yeast culture (1) in close proximity to the signal emitters such as a metal wire or tube capable of transmitting EMFs. The metal wire or tube can be made of red copper, and be placed inside the container  
25 (2), reaching as deep as 3-30 cm. For example, if the fluid in the container (2) has a depth of 15-20 cm, 20-30 cm, 30-50 cm, 50-70 cm, 70-100 cm, 100-150 cm or 150-200 cm, the metal wire can be 3-5 cm, 5-7 cm, 7-10 cm, 10-15 cm, 15-20 cm, 20-30 cm and 25-30 cm from the bottom of the container (2), respectively. The number of electrode wires used depends on the volume of the culture as well as the  
30 diameter of the wires. The number of metal wires/tubes used can be from 1 to 10 (e.g., 2 to 3). It is recommended, though not mandated, that for a culture having a volume up to 10 L, metal wires/tubes having a diameter of 0.5 to 2.0 mm be used.

For a culture having a volume between 10 L and 100 L, metal wires/tubes having a diameter of 3.0 to 5.0 mm can be used. For a culture having a volume in the range of 100-1000 L, metal wires/tubes having a diameter of 6.0 to 15.0 mm can be used. For a culture having a volume greater than 1000 L, metal wires/tubes having a  
5 diameter of 20.0 to 25.0 mm can be used.

In one embodiment, the electric field is applied by electrodes submerged in the culture (1). In this embodiment, one of the electrodes can be a metal plate placed on the bottom of the container (2), and the other electrode can comprise a plurality of electrode wires evenly distributed in the culture (1) so as to  
10 achieve even distribution of the electric field energy. The number of electrode wires used depends on the volume of the culture as well as the diameter of the wires.

### **III. Culture Media**

Culture media useful in this invention contain sources of nutrients  
15 assimilable by yeast cells. Complex carbon-containing substances in a suitable form, such as carbohydrates (e.g., sucrose, glucose, fructose, dextrose, maltose, xylose, cellulose, starches, etc.) and coal, can be the carbon sources for yeast cells. The exact quantity of the carbon sources utilized in the medium can be adjusted in accordance with the other ingredients of the medium. In general, the amount of  
20 carbohydrates varies between about 0.1% and 10% by weight of the medium and preferably between about 0.1% and 5% (e.g., about 2%). These carbon sources can be used individually or in combination. Amino acid-containing substances in suitable form (e.g., beef extract and peptone) can also be added individually or in combination. In general, the amount of amino acid containing substances varies  
25 between about 0.1% and 0.5% by weight of the medium and preferably between about 0.1% and 0.3% (e.g., about 0.25%). Among the inorganic salts which can be added to the culture medium are the customary salts capable of yielding sodium, potassium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are  $(\text{NH}_4)_2\text{HPO}_4$ ,  $\text{KH}_2\text{PO}_4$ ,  $\text{K}_2\text{HPO}_4$ ,  $\text{CaCO}_3$ ,  
30  $\text{MgSO}_4$ ,  $\text{NaCl}$ , and  $\text{CaSO}_4$ .

### **IV. Electromagnetic Activation of Yeast Cells**

To activate or enhance the ability of yeast cells to produce substances beneficial for the treatment of nephrotic syndrome (e.g., decreasing urinary protein and/or increasing serum protein levels), these cells can be activated by being cultured in an appropriate medium under sterile conditions at 20°C-38°C, preferably at 28-32°C (e.g., 30°C) for a sufficient amount of time, e.g., 5-200 hours (e.g., 6-16, 10-20, 27-37 and 31-41 hours), in an alternating electric field or a series of alternating electric fields as described above.

An exemplary culture medium is made by mixing 1000 ml of distilled water with 18 g of mannitol, 40 µg of vitamin B<sub>12</sub>, 30 µg of vitamin E, 30 µg of vitamin H, 35 ml of fetal bovine serum, 0.20 g of KH<sub>2</sub>PO<sub>4</sub>, 0.25 g of MgSO<sub>4</sub>•7H<sub>2</sub>O, 0.3 g of NaCl, 0.2 g of CaSO<sub>4</sub>•2H<sub>2</sub>O, 4.0 g of CaCO<sub>3</sub>•5H<sub>2</sub>O, and 2.5 g of peptone.

An exemplary set-up of the culturing process is depicted in Fig. 1. Untreated yeast cells are added to a culture medium at 1x10<sup>8</sup> cells per 1000 ml of the culture medium. The yeast cells may be *Saccharomyces cerevisiae* Hansen AS2.502, or may be selected from any of the strains listed in Table 1. An exemplary activation process of the yeast cells involves the following sequence: the yeast cells are grown in the culture medium for 23-33 hours (e.g., 28 hours) at 28-32°C and then exposed to (1) an alternating electric field having a frequency of 10156 MHz and a field strength in the range of 325-355 mV/cm (e.g., 332 mV/cm) for 6-16 hours (e.g., 11 hours); (2) then to an alternating electric field having a frequency of 10185 MHz and a field strength in the range of 400-440 mV/cm (e.g., 416 mV/cm) for 31-41 hours (e.g., 36 hours); (3) then to an alternating electric field having a frequency of 12107 MHz and a field strength in the range of 430-470 mV/cm (e.g., 443 mV/cm) for 27-37 hours (e.g., 32 hours); (4) then to an alternating electric field having a frequency of 12687 MHz and a field strength in the range of 340-370 mV/cm (e.g., 353 mV/cm) for 31-41 hours (e.g., 36 hours); and (5) finally to an alternating electric field having a frequency of 12698 MHz and a field strength in the range of 285-305 mV/cm (e.g., 296 mV/cm) for 10-20 hours (e.g., 15 hours). The activated yeast cells are then recovered from the culture medium by various methods known in the art, dried (e.g., by lyophilization) and

stored at about 4°C in powder form. The resultant yeast powder preferably contains no less than  $10^{10}$  cells/g activated yeast.

Subsequently, the activated yeast cells can be evaluated for their ability to treat nephrotic syndrome using standard methods known in the art, such as those described in Section VII.

#### V. Acclimatization of Yeast Cells To the Gastric Environment

Because the activated yeast cells of this invention must pass through the stomach before reaching the small intestine, where the effective components are released from these yeast cells, it is preferred that these yeasts be cultured under acidic conditions so as to acclimatize the cells to the gastric juice. This acclimatization process results in better viability of the yeast cells in the acidic gastric environment.

To achieve this, the yeast powder containing activated yeast cells can be mixed with a highly acidic acclimatizing culture medium at 10 g (containing more than  $10^{10}$  activated cells per gram) per 1000 ml. The yeast mixture can then be cultured first in the presence of an alternating electric field having a frequency of 12687 MHz and a field strength in the range of 360-390 mV/cm (e.g., 364 mV/cm) at about 28 to 32°C for 36-48 hours (e.g., 44 hours). The resultant yeast cells can then be further incubated in the presence of an alternating electric field having a frequency of 12698 MHz and a field strength in the range of 285-315 mV/cm (e.g., 296 mV/cm) at about 28 to 32°C for 16-28 hours (e.g., 20 hours). The resulting acclimatized yeast cells are then recovered from the culture medium by various methods known in the art and are dried and stored either in powder form ( $\geq 10^{10}$  cells/g) at room temperature or in vacuum at 0-4°C.

An exemplary acclimatizing culture medium is made by mixing 700 ml fresh pig gastric juice and 300 ml wild Chinese hawthorn extract. The pH of acclimatizing culture medium is adjusted to 2.5 with 0.1 M hydrochloric acid (HCl) and 0.2 M potassium hydrogen phthalate ( $C_6H_4(COOK)COOH$ ). The fresh pig gastric juice is prepared as follows. At about 4 months of age, newborn Holland white pigs are sacrificed, and the entire contents of their stomachs are retrieved and mixed with 2000 ml of water under sterile conditions. The mixture is then allowed to stand for 6 hours at 4°C under sterile conditions to precipitate food debris. The



supernatant is collected for use in the acclimatizing culture medium. To prepare the wild Chinese hawthorn extract, 500 g of fresh wild Chinese hawthorn is dried under sterile conditions to reduce water content ( $\leq 8\%$ ). The dried fruit is then ground ( $\geq 20$  mesh) and added to 1500 ml of sterilized water. The hawthorn slurry is allowed to stand for 6 hours at  $4^{\circ}\text{C}$  under sterile conditions. The hawthorn supernatant is collected to be used in the acclimatizing culture medium.

## VI. Manufacture of Yeast Compositions

To prepare the yeast compositions of the invention, an apparatus depicted in Fig. 2 or an equivalent thereof can be used. This apparatus includes three containers, a first container (A), a second container (B), and a third container (C), each equipped with a pair of electrodes (4). One of the electrodes is a metal plate placed on the bottom of the containers, and the other electrode comprises a plurality of electrode wires evenly distributed in the space within the container to achieve even distribution of the electric field energy. All three pairs of electrodes are connected to a common signal generator.

The culture medium used for this purpose is a mixed fruit extract solution containing the following ingredients per 1000 L: 300 L of wild Chinese hawthorn extract, 300 L of jujube extract, 300 L of *Schisandra chinensis* (Turez) Baill seeds extract, and 100 L of soy bean extract. To prepare hawthorn, jujube and *Schisandra chinensis* (Turez) Baill seeds extracts, the fresh fruits are washed and dried under sterile conditions to reduce the water content to no higher than 8%. One hundred kilograms of the dried fruits are then ground ( $\geq 20$  mesh) and added to 400 L of sterilized water. The mixtures are stirred under sterile conditions at room temperature for twelve hours, and then centrifuged at 1000 rpm to remove insoluble residues. To make the soy bean extract, fresh soy beans are washed and dried under sterile conditions to reduce the water content to no higher than 8%. Thirty kilograms of dried soy beans are then ground into particles of no smaller than 20 mesh, and added to 130 L of sterilized water. The mixture is stirred under sterile conditions at room temperature for twelve hours and centrifuged at 1000 rpm to remove insoluble residues. Once the mixed fruit extract solution is prepared, it is autoclaved at  $121^{\circ}\text{C}$  for 30 minutes and cooled to below  $40^{\circ}\text{C}$  before use.

One thousand grams of the activated yeast powder prepared as described above (Section V, *supra*) is added to 1000 L of the mixed fruit extract solution, and the yeast solution is transferred to the first container (A) shown in Fig. 2. The yeast cells are then cultured in the presence of an alternating electric field having a frequency of 12687 MHz and a field strength of about 460-500 mV/cm (e.g., 487 mV/cm) at 28-32°C under sterile conditions for 27-37 hours (e.g., 32 hours). The yeast cells are further incubated in an alternating electric field having a frequency of 12698 MHz and a field strength of 410-450 mV/cm (e.g., 435 mV/cm). The culturing continues for 7-17 hours (e.g., 12 hours).

10 The yeast culture is then transferred from the first container (A) to the second container (B) (if need be, a new batch of yeast culture can be started in the now available the first container (A)), and subjected to an alternating electric field having a frequency of 12687 MHz and a field strength of 480-520 mV/cm (e.g., 507 mV/cm) for 19-29 hours (e.g., 24 hours). Subsequently the frequency and field strength of the electric field are changed to 12698 MHz and 440-480 mV/cm (e.g., 456 mV/cm), respectively. The culturing process continues for 7-17 hours (e.g., 12 hours).

The yeast culture is then transferred from the second container (B) to the third container (C), and subjected to an alternating electric field having a frequency of 12687 MHz and a field strength of 360-390 mV/cm (e.g., 373 mV/cm) for 19-29 hours (e.g., 24 hours). Subsequently the frequency and field strength of the electric field are changed to 12698 MHz and 320-350 mV/cm (e.g., 332 mV/cm), respectively. The culturing continues for 7-17 hours (e.g., 12 hours).

25 The yeast culture from the third container (C) can then be packaged into vacuum sealed bottles, each having 30-50 ml or 100 ml of the yeast culture, for use as a dietary supplement, e.g., health drinks, or medication in the form of pills, powder, etc. If desired, the final yeast culture can also be dried within 24 hours and stored in powder form. The dietary supplement can be taken orally three times daily at 30 ml per dose for a three-month period, preferably before meals.

30 In some embodiments, the compositions of the invention can also be administered intravenously or peritoneally in the form of a sterile injectable preparation. Such a sterile preparation can be prepared as follows. A sterilized

health drink composition is first treated under ultrasound ( $\geq 18000$  Hz) for 10 minutes and then centrifuged at 4355 rpm for another 10 minutes. The resulting supernatant is adjusted to pH 7.2-7.4 using 1 M NaOH and subsequently filtered through a membrane (0.22  $\mu\text{m}$  for intravenous injection and 0.45  $\mu\text{m}$  for peritoneal  
5 injection) under sterile conditions. The resulting sterile preparation is submerged in a 35-38°C water bath for 30 minutes before use. In other embodiments, the compositions of the invention may also be formulated with pharmaceutically acceptable carriers to be orally administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, suspensions or solutions.

10 The yeast compositions of the present invention are derived from yeasts used in food and pharmaceutical industries. The yeast compositions are thus devoid of side effects associated with many pharmaceutical compounds.

## **VII. Examples**

In order that this invention be more fully understood, the following  
15 examples are set forth. These examples are for the purpose of illustration only and are not to be construed as limiting the scope of the invention in any way.

The activated yeast compositions used in the following examples were prepared as described above, using *Saccharomyces cerevisiae* Hansen AS2.502, cultured in the presence of an alternating electric field having the electric  
20 field frequency and field strength exemplified in the parentheses following the recommended ranges listed in Section IV, *supra*. Control (i.e., untreated) yeast compositions were those prepared in the same manner as described in Section VI, *supra*, except that the yeast cells were cultured in the absence of EMFs. Unless otherwise specified, all compositions of interest were administered to the animals  
25 by intragastric feeding.

### **Example 1: Effect of Treatment on Proteinuria**

To test the ability of the activated yeast compositions to reduce the level of urinary protein, sixty healthy Wistar rats with average weight of about 200-220 g (4-7 months old, half of them male and the other half female) were chosen  
30 and males and females were kept in separate cages. Each rat was injected intravenously with bovine serum albumin (BSA; at 350 mg/kg body weight) in the marginal ear vein to induce excess secretion of protein in the urine (proteinuria).

After the injection, each rat was given normal feed for seven days. Urine samples were collected from the fine cancellated base of metabolic cages, and the amount of protein in the samples was determined by hot acetic acid method. Forty rats were selected for further study from those showing proteinuria, i.e., less than 0.5 mg/24  
5 hours, and randomly divided into four equal groups, designated as AY, NY, CK1 and CK2.

Subsequently, a composition of interest was administered twice daily to each of the four groups of rats for eight weeks. Rats in the AY, NY and CK1 groups received the 1.0 ml/100 g body weight of the activated yeast  
10 composition, the control yeast composition and saline, respectively. Rats in the CK2 group received 0.25 mg/100 g body weight of pednisone (metacortandiacin). Urine samples were collected for 24 hours on the last day of the fourth week as well as on the last day of the eighth week.

The amount of urinary protein was determined by sulfosalicylic acid  
15 turbidimetry. The volume of each urine sample was first measured (ml). Five milliliters of each sample was then taken out and centrifuged at 3000 rpm. One milliliter of the supernatant was mixed with 3 ml of 30 mg/ml sulfosalicylic acid in a test tube. In the control tube, 1 ml saline was mixed with 3 ml of 30 mg/ml sulfosalicylic acid. Ten minutes later, the absorption of the sample test tube was  
20 measured at 620 nm against the control tube. The amount of urinary protein (per 100 ml) was determined based on a protein standard curve.

The protein standard curve was created according to the following procedure. The amount of protein in fresh sera free of unhemolysis and unbilirubin were determined by commonly used Kjeldahl's method. The fresh sera were then  
25 diluted to 4 mg/ml with saline. Seven mixtures were prepared according to Table 2. Absorption was determined for each mixture containing diluted sera against the control mixture, which had no serum. The protein standard curve was thus created with protein concentrations and their corresponding absorption.

Table 2.

	Tube #	1	2	3	4	5	6	Control
	4 mg/ml Serum (ml)	0.0125	0.025	0.05	0.1	0.2	0.4	0
5	Saline (ml)	0.9875	0.975	0.95	0.9	0.8	0.6	1.0
	30 mg/ml Sulfosalicylic acid (ml)	3.0	3.0	3.0	3.0	3.0	3.0	3.0
10	Protein Conc. (mg%)	5.0	10.0	20.0	40.0	80.0	160.0	0.0

The amount urinary protein (mg) in 100 ml urine samples was calculated by multiplying urinary protein concentration (mg%) by the total urine volume in the 24-hour collection period (ml) and divided by 100 and summarized in Table 3.

15 Table 3. The Effect of Treatment on Urinary Protein secretion

Group	Urinary Protein (mg in 100 ml urine sample) in a 24-hour Period	
	4-Week Treatment	8-Week Treatment
CK1	7.21±2.34	7.43±2.52
CK2	4.57±1.42	4.22±1.34
NY	7.33±2.45	7.41±2.42
20 AY	0.62±0.21	0.34±0.14

The results in Table 3 show that the activated yeast composition was more effective in reducing the amount of urinary protein than the control yeast composition, saline or pednisone.

### **Example 2: Effect of Treatment on Serum Protein**

To test the ability of the activated yeast compositions to reduce the level of urinary protein, sixty healthy Wistar rats with average weight of about 200-220 g (4-6 months old, half of them male and the other half female) were chosen and prepared as described in Example 1.

Subsequently, a composition of interest was administered twice daily to each of the four groups of rats for six weeks. Rats in the AY, NY and CK1 groups received 1.0 ml/100 g body weight of the activated yeast composition, the control yeast composition and saline, respectively. Rats in the CK2 group received 0.2 mg/100 g body weight of pednisone. Six weeks later, the rats were anesthetized with ether and blood samples were collected from the carotid artery and centrifuged at 3000 rpm. The amount of protein in the supernatant (serum protein) was determined.

To determine the amount of serum protein, 50  $\mu$ l of the supernatant and standard serum protein were added into two separate tubes. Four milliliters of allophanamide (biuret) was added to each tube and mixed with the samples. The mixtures were placed in water bath at 37°C for 10 minutes and measured for absorption at 546 nm. The concentration of serum protein was calculated according to the following formula:

$$[\text{Serum Protein}] = [\text{absorption for the testing sample} / \text{absorption for the standard}] \times [\text{standard serum protein}] \text{ (g/dl)}.$$

The results are summarized in Table 4.

Table 4. The Effect of Treatment on Serum Protein

Group	Treatment	Serum Protein (g/dl)
CK1	Saline (1.0 ml/100 g)	4.72 $\pm$ 2.33
CK2	Pednisone (0.2 mg/100 g)	6.41 $\pm$ 3.32
NY	Control Yeast Composition (1.0 ml/100 g)	4.64 $\pm$ 2.44
AY	Activated Yeast Composition (1.0 ml/100 g)	8.86 $\pm$ 3.26

The results in Table 4 show that unlike the control yeast composition, saline or prednisone, the activated yeast composition was effective in increasing serum protein level in subjects with hypoalbuminemia.

While a number of embodiments of this invention have been set  
5 forth, it is apparent that the basic constructions may be altered to provide other  
embodiments which utilize the compositions and methods of this invention.